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A slow-release system of bacterial cellulose gel and nanoparticles for hydrophobic active ingredients

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ABSTRACT

A combination of bacterial cellulose (BC) gel and amphiphilic block copolymer nanoparticles was investigated as a drug delivery system (DDS) for hydrophobic active ingredients. Poly(ethylene oxide)-*b*-poly(caprolactone) (PEO-*b*-PCL) and retinol were used as the block copolymer and hydrophobic active ingredient, respectively. The BC gel was capable of incorporating copolymer nanoparticles and releasing them in an acetic acid–sodium acetate buffer solution (pH 5.2) at 37 °C. The percentage of released copolymer reached a maximum value of approximately 60% after 6 h and remained constant after 24 h. The percentage of retinol released from the copolymer-containing BC gel reached a maximum value at 4 h. These results show that the combination of BC gel and nanoparticles is a slow-release system that may be useful in the cosmetic and biomedical fields for skin treatment and preparation.

1. Introduction

Bacterial cellulose (BC) gel is a unique hydrogel consisting of greater than 99% (w/w) water by weight. BC gels have a three-dimensional network structure of ultrafine fiber made from pure cellulose (Brown, 1996; Iguchi et al., 2000; Ross et al., 1991) and have various useful properties, such as softness, translucence, and good biocompatibility and water retention capacity (Klemm et al., 2005; Miyamoto et al., 1989; Okiyama et al., 1993). BC gels have been extensively investigated as potential soft materials for use in biomedical fields (Fu et al., 2013; Shah et al., 2013). In skin tissue repair, the advantages of BC, such as biocompatibility, conformability, elasticity, transparency, the ability to maintain a moist environment in the wound, and the ability to absorb exudate during the inflammatory phase, confer great potential for application in wound healing systems. In cosmetic applications, BC gels offer good biocompatibility, conformability, elasticity, and transparency, as well as the ability to maintain a moist environment for active ingredients. BC gels are commercially available as a cosmetic facial mask in Japan. BC facial masks adhere well to the face and retain moisture better than normal cellulose sheets, because BC consists of very thin nano-fibers. However, the incorporation of hydrophobic active ingredients into BC gels remains a challenging task. New methods to effectively incorporate hydrophobic molecules would allow their application in BC gel instead of in oily cosmetic products such as facial creams; therefore, the development of such methods will be useful in the cosmetics market and in biomedical applications.

Amphiphilic block copolymer systems are used to incorporate hydrophobic molecules, and these copolymers have the ability to self-assemble into well-organized structures, such as nanoparticles, micelles, and vesicles, in aqueous solutions. Micelles can have a diameter of 10–200 nm, but most have a diameter ranging from 10 to 100 nm. Polymers typically have molecular weights ranging from several hundred to several tens of thousands. Particle size depends on sample preparation conditions and the type of copolymer, as well as its components, molecular weight, and organic solvent composition (Aliabadi et al., 2007). Micelles can be produced by methods such as dialysis (Allen et al., 1999) and co-solvent evaporation (Aliabadi et al., 2005b). Nanoparticles produced from biocompatible and biodegradable copolymers have shown great potential as carriers for active ingredients in cosmetic

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and pharmaceutical applications. Poly(ethylene oxide)-*b*-poly (caprolactone) (PEO-*b*-PCL) is composed of biocompatible and biodegradable synthetic polymers and is one of the most extensively studied copolymer systems for nanoparticle formation (Wei et al., 2009). The PEO-*b*-PCL copolymer forms nano-sized micelles, in which PEO is the hydrophilic shell-forming block and PCL is the hydrophobic core-forming block. Many researchers have reported encapsulation of hydrophobic active ingredients into PEO-*b*-PCL nanoparticles (Aliabadi et al., 2005a; Kim et al., 1998; Mahmud and Lavasanifar, 2005; Shuai et al., 2004). The ultimate objective of such studies was the development of a drug delivery system (DDS).

PEO (poly(ethylene glycol) (PEG)), the hydrophilic shell-forming block of PEO-*b*-PCL nanoparticles, has high affinity for cellulose (Numata et al., 2009). Therefore, we hypothesized that PEO-*b*-PCL nanoparticles can be introduced into BC gel, which can then be used to encapsulate hydrophobic molecules. If the BC gel can release PEO-*b*-PCL nanoparticles and the encapsulated hydrophobic molecules, then the system can be utilized in topical compound preparations in the cosmetic and the biomedical fields. In this study, we designed a DDS by combining BC gels with a nanoparticle-encapsulated hydrophobic active ingredient. We used PEO-*b*-PCL and retinol as the block copolymer and hydrophobic active ingredient, respectively. Retinol, vitamin A alcohol, is extensively used in the pharmaceutical and cosmetic industries and is a well-known anti-aging ingredient. The PEO-*b*-PCL nanoparticle-containing BC gel was investigated for the presence of BC containing nanoparticles by Fourier transform infrared (FT-IR) spectra and field-emission gum scanning electron microscopy (FEG-SEM). The released nanoparticles indicated that the PEO-*b*-PCL nanoparticle-containing BC gel can be utilized as a DDS, and the retinol release indicated that packaged retinol can be released from the BC gel; however, the DDS must be improved to support practical use. Our results show that the DDS described herein may be useful in topical preparations in the cosmetic and biomedical fields.

2. Material and methods

2.1. Materials

Three types of PEO-*b*-PCL block copolymers were purchased from Polymer Source, Inc. (Quebec, Canada). The molecular weights (M_w) of PEO and PCL in the three PEO-*b*-PCL block copolymers were 5000 and 5000, 5000 and 10,000, and 2000 and 11,500, respectively. The M_w/number average molecular weight (M_n) ratios of the three types of PEO-*b*-PCL copolymers were 1.20, 1.26, and 1.20. Retinol was purchased from Sigma–Aldrich Co. LLC (St. Louis, MO, USA).

2.2. Preparation of PEO-b-PCL nanoparticles and retinol-loaded PEO-b-PCL nanoparticles

PEO-*b*-PCL nanoparticles were prepared by co-solvent evaporation (Aliabadi et al., 2005b). PEO-*b*-PCL (10 mg) dissolved in acetone (0.5 mL) was added in a drop-wise manner (5.08 mL/h) to Milli-Q water (1 mL) with stirring. The remaining acetone was removed by evaporation at room temperature under vacuum.

Retinol-loaded PEO-*b*-PCL nanoparticles were prepared by co-solvent evaporation as described above except for dissolving retinol and PEO-*b*-PCL in acetone. PEO-*b*-PCL (10 mg) and retinol (3, 6, or 15 mg) dissolved in acetone (0.5 mL) were added in a drop-wise manner (5.08 mL/h) to Milli-Q water (1 mL) with stirring. The remaining acetone was removed by evaporation at room temperature under vacuum. The obtained nanoparticle suspension was centrifuged at 5673 × *g* for 5 min to remove the retinol precipitate from the outside of the nanoparticles.

2.3. Preparation of BC gel containing PEO-b-PCL nanoparticles

BC gels were biosynthesized using *Gluconacetobacter xylinus* ATCC 12733 in Hestrin and Schramm medium for 3 weeks under static conditions (Hestrin and Schramm, 1954). Subsequently, the gels were purified using running water for 2 days, deproteinized in 0.5% (w/w) NaOH solution, neutralized in 0.5% (w/w) acetic acid solution, and washed with distilled water.

BC gels containing PEO-*b*-PCL nanoparticles were prepared by diffusion of nanoparticles into the gels. BC gels (sample size = 2 cm × 2 cm, thickness = 2 mm) were immersed in the PEO-*b*-PCL nanoparticle suspension (10 mg/mL) for 1 day at room temperature. BC gels containing retinol-loaded PEO-*b*-PCL nanoparticles were prepared by immersion of the gels in the PEO-*b*-PCL nanoparticle suspension (10 mg/mL, initial retinol/copolymer weight ratio of 0.15) for 1 day at 4 °C.

2.4. Characterization of PEO-b-PCL nanoparticles

2.4.1. Dynamic light scattering

The hydrodynamic radius of the obtained nanoparticles in aqueous media and acetic acid-sodium acetate buffer (pH 5.2) was measured using dynamic light scattering (DLS) at 25 °C. The hydrodynamic diameter was calculated from the Stokes–Einstein relation. The instrument was an ALV/CGS-8FS/N069 apparatus (ALV GmbH, Langen, Germany) equipped with an ALV/LSE-5004 multiple tau digital correlator with a 125 ns initial sampling time (ALV GmbH) and a 35 mW red helium–neon linearly polarized laser operating at a wavelength of 632.8 nm (JDS Uniphase Corporation, Milpitas, CA, USA). The concentration of the block copolymer was 1 mg/mL, and it was diluted using distilled water or acetic acid-sodium acetate buffer (pH 5.2).

2.4.2. Transmission electron microscopy

The morphology of the PEO-*b*-PCL nanoparticles was observed under a Philips CM200 microscope (Royal Philips, Amsterdam, Netherlands) operated at 120 kV. PEO-*b*-PCL nanoparticles in aqueous media (4 μL) or acetic acid–sodium acetate buffer (pH 5.2) (4 μL), with a block copolymer concentration of 1 mg/mL, were dropped on a glow-discharge carbon-coated copper grid. Next, 2% (w/v) uranyl acetate negative stain (4 μL) was added and allowed to dry completely.

2.4.3. Retinol encapsulation

The amount of retinol in the PEO-*b*-PCL nanoparticles was determined using a Perkin-Elmer Lambda 10 UV/Vis spectrophotometer (PerkinElmer, Inc., Waltham, MA, USA) at 325 nm. The retinol-loaded PEO-*b*-PCL nanoparticle suspension was dissolved in acetonitrile to extract the retinol from the nanoparticles. The retinol encapsulation efficiency was calculated using the following equation:

$$\text{Encapsulation (\%)} = \frac{\text{Loaded retinol(mg)}}{\text{Initial retinol(mg)}}$$

2.4.4. Stability of encapsulated retinol

The retinol-loaded PEO(5000)-*b*-PCL(5000) nanoparticle suspension was diluted so that the concentrations of block copolymer and retinol were 5 mg/mL and 1.5 mg/mL, respectively. Next, the acetic acid–sodium acetate buffer (pH 5.2) (19 mL) and the